Fig. 1 3nt Overhang elongation (RSPS Variant 1)

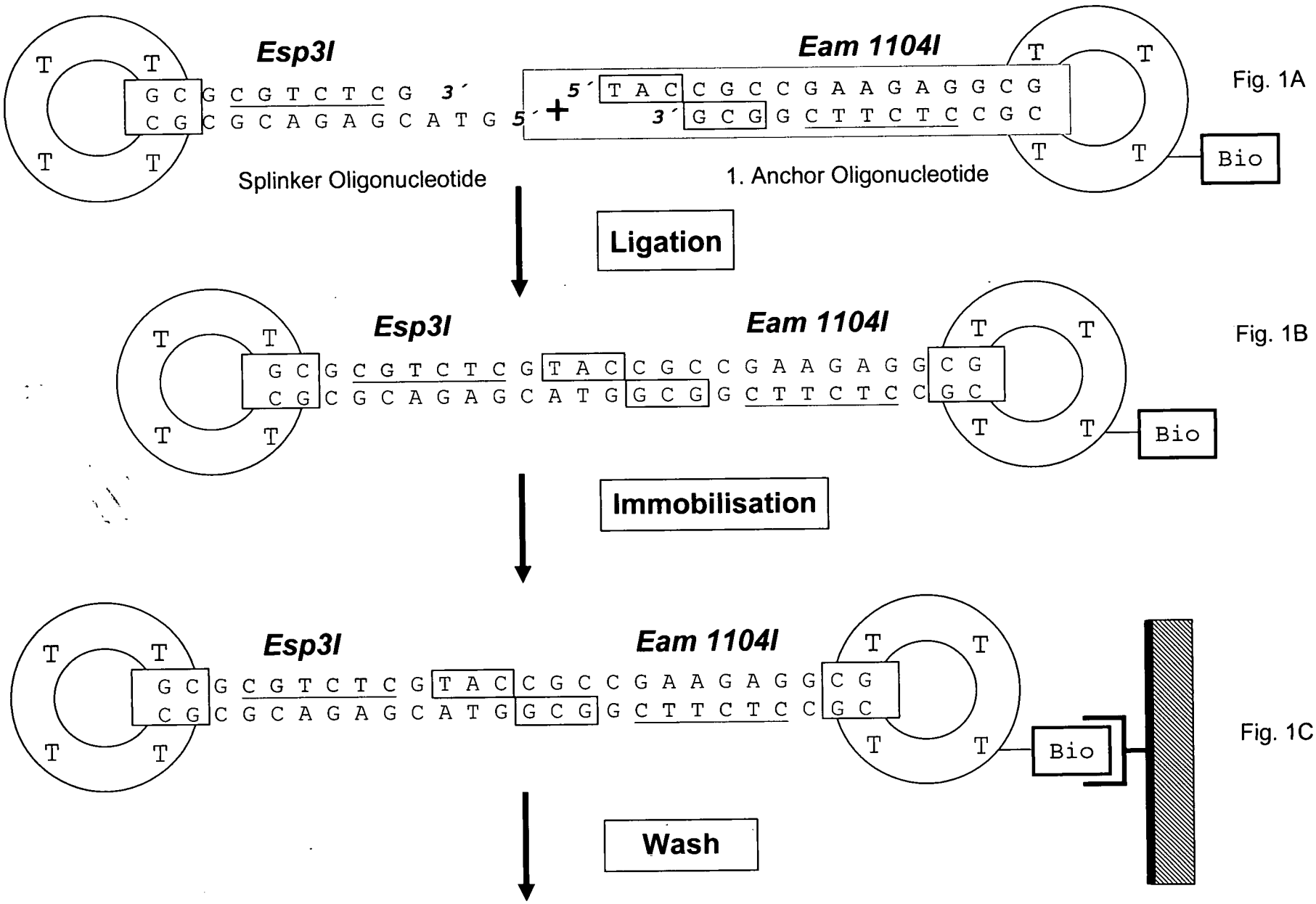


Fig. 1A

Fig. 1B

Fig. 1C

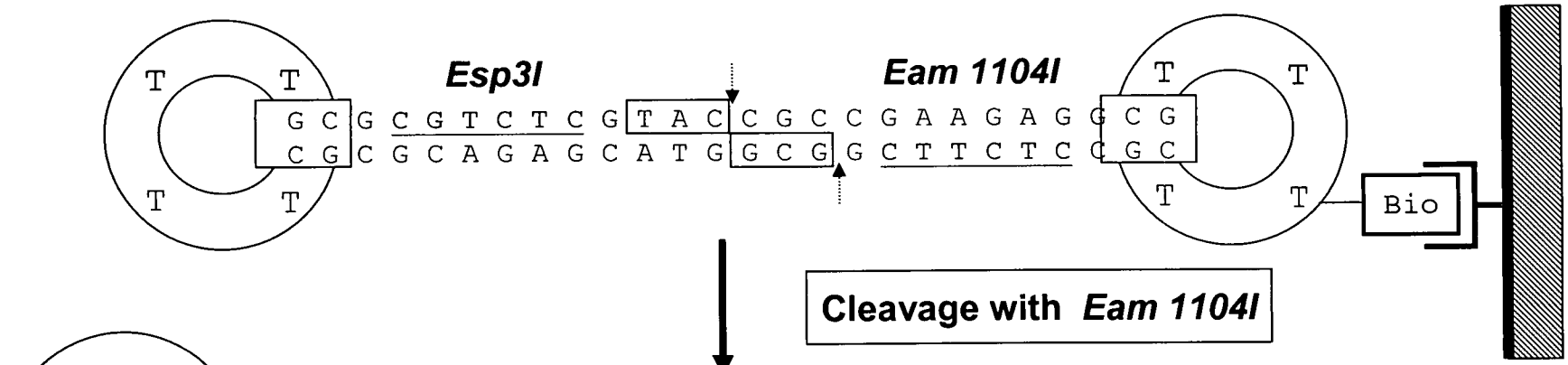


Fig. 1D

Cleavage with *Eam 1104I*

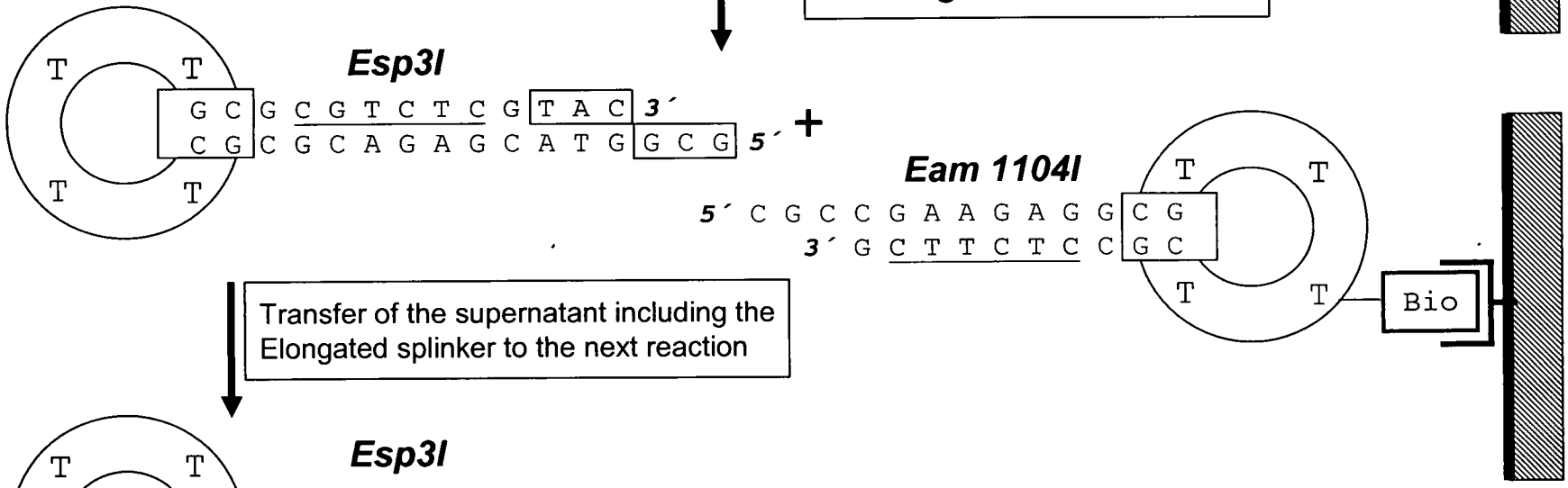


Fig. 1E

Transfer of the supernatant including the Elongated splinker to the next reaction

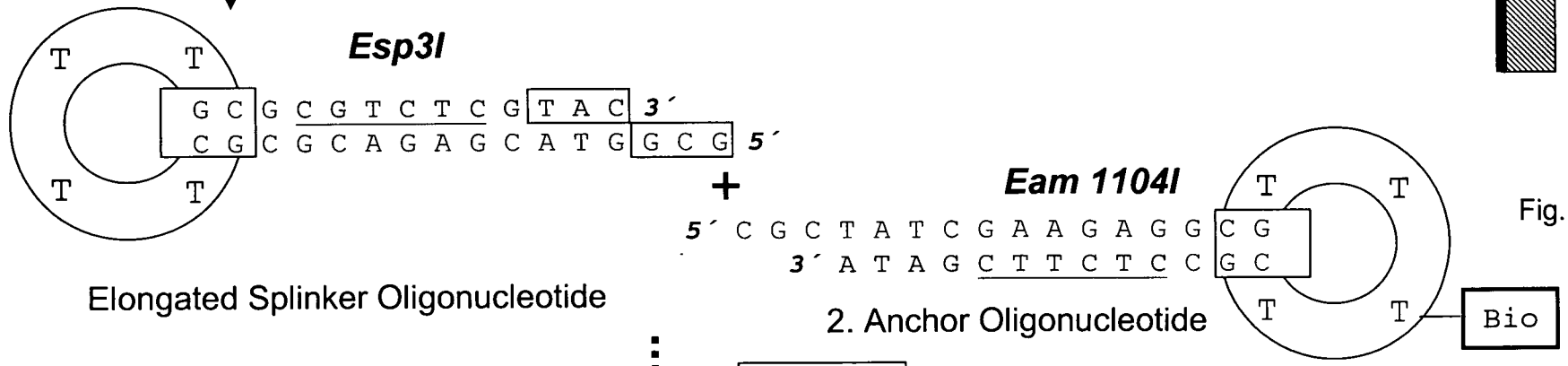


Fig. 1F

Elongated Splinker Oligonucleotide

2. Anchor Oligonucleotide

Ligation

10/531556

Fig. 2 1nt Overhang elongation (RSPS Variant 1)

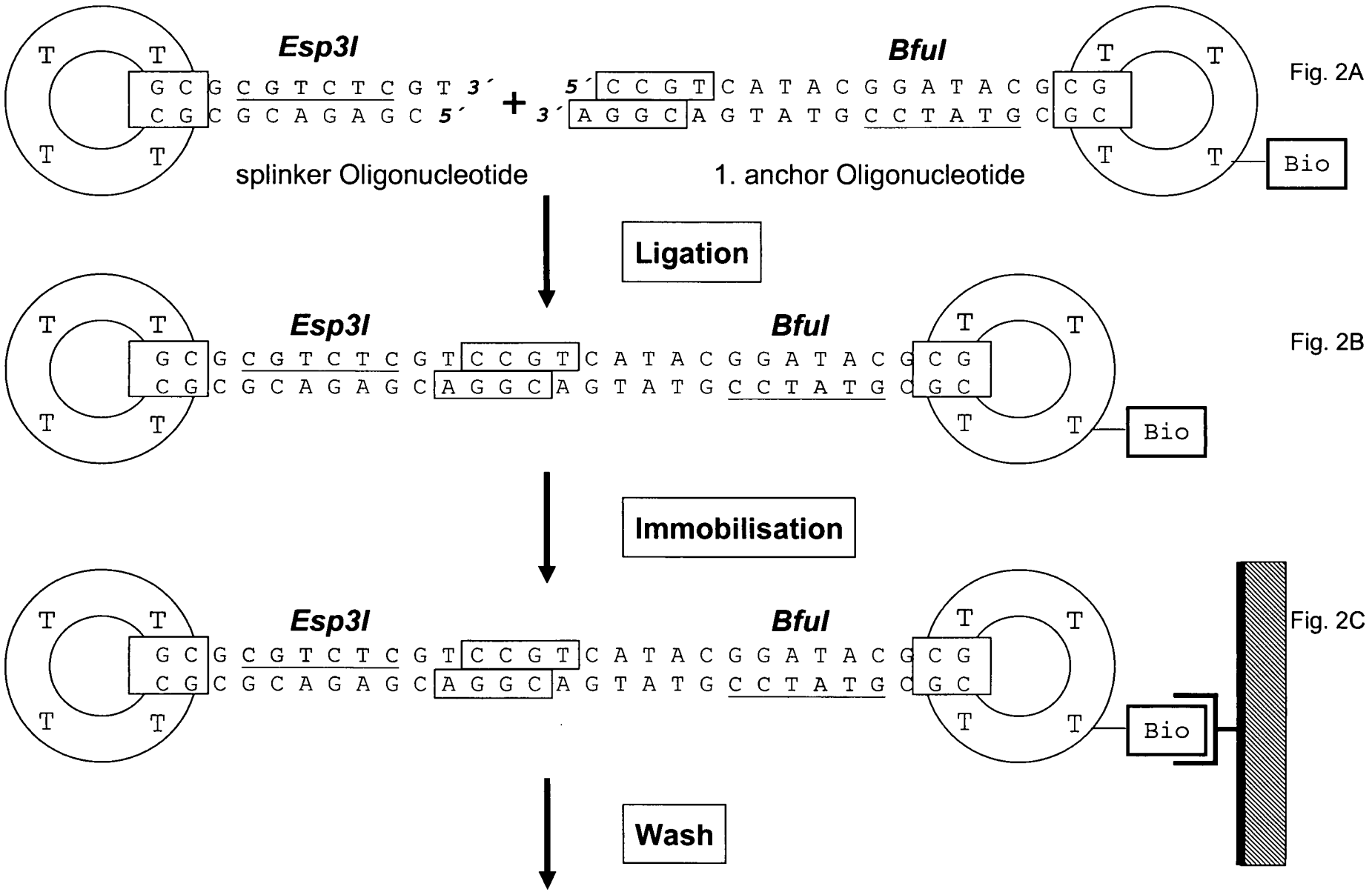


Fig. 2A

Fig. 2B

Fig. 2C

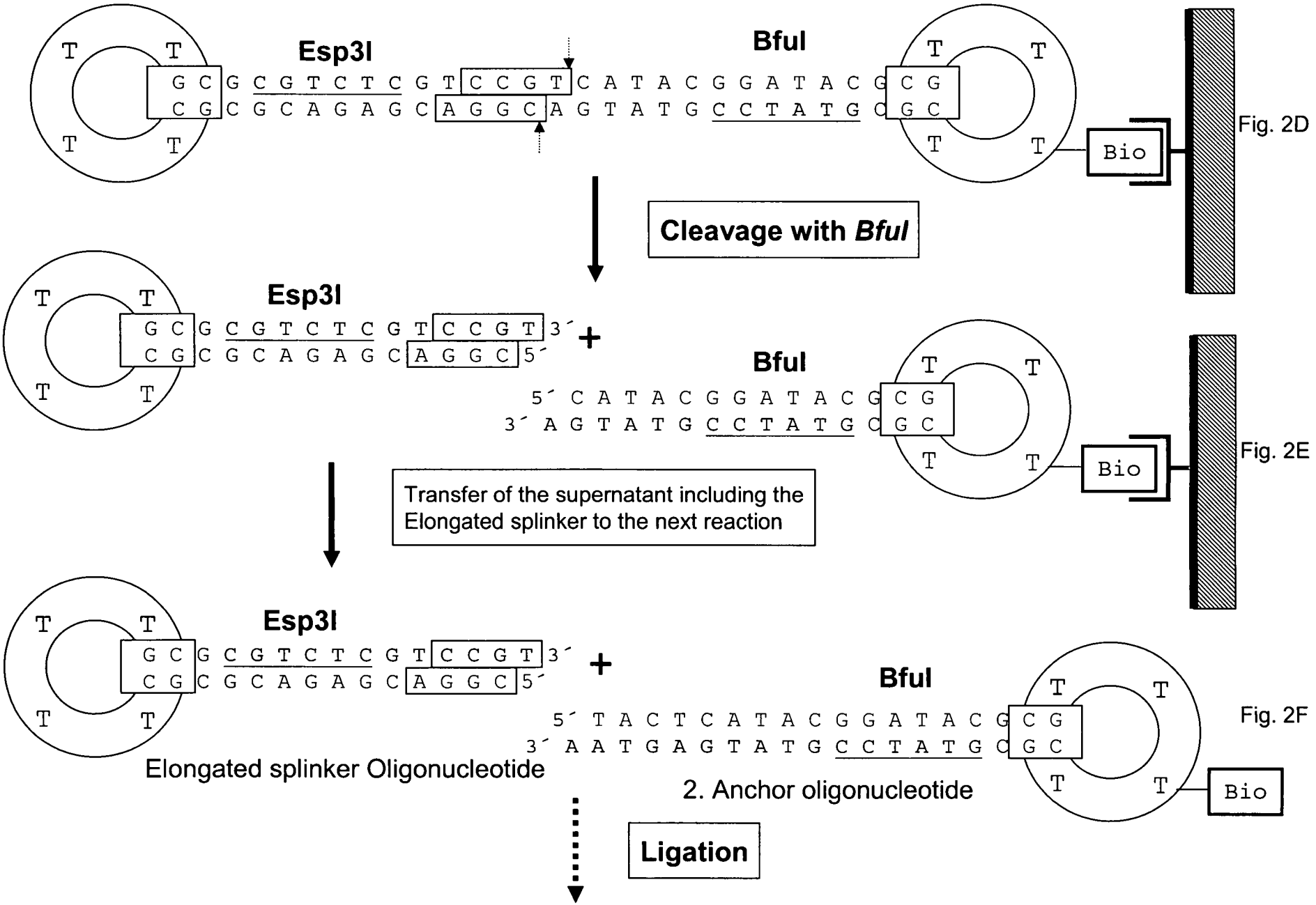


Fig. 2D

Fig. 2E

Fig. 2F

Fig. 3 3nt Overhang elongation (RLPS Variant 1)

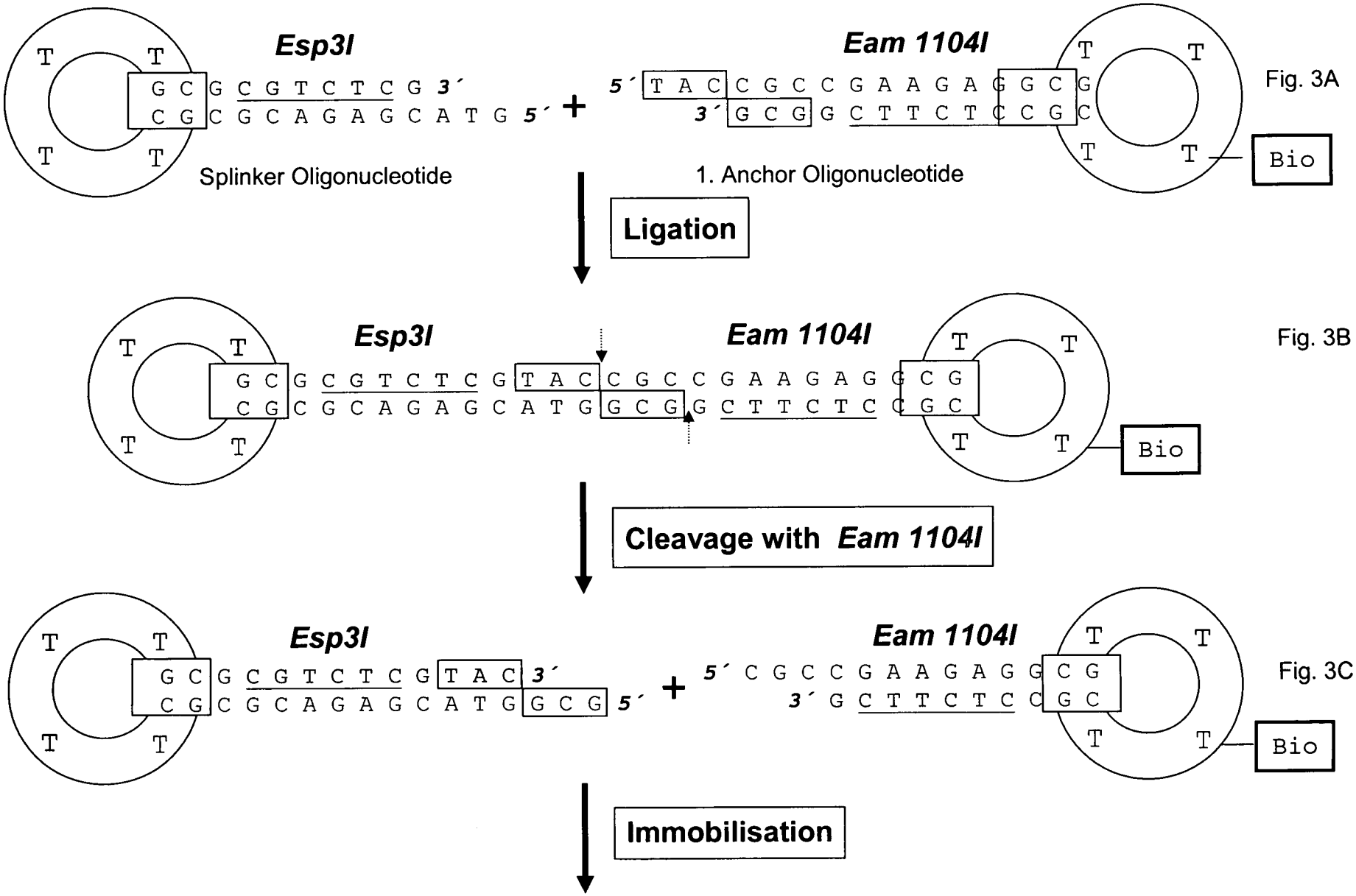


Fig. 3A

Fig. 3B

Fig. 3C

Fig. 3D

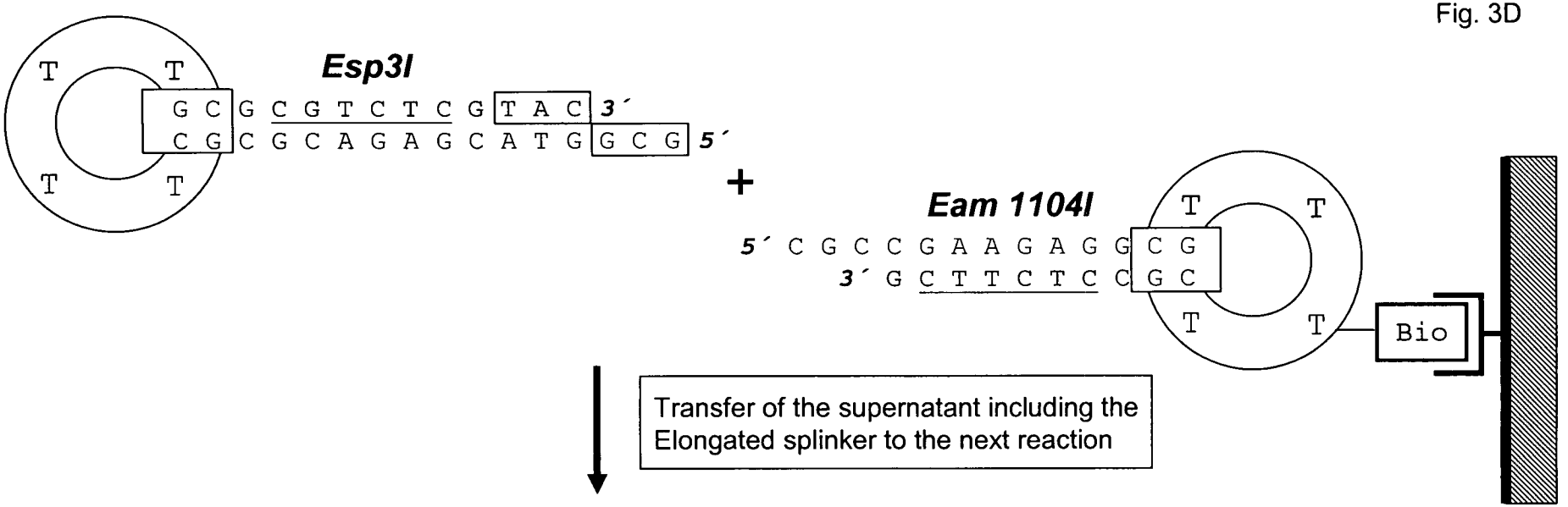


Fig. 3E

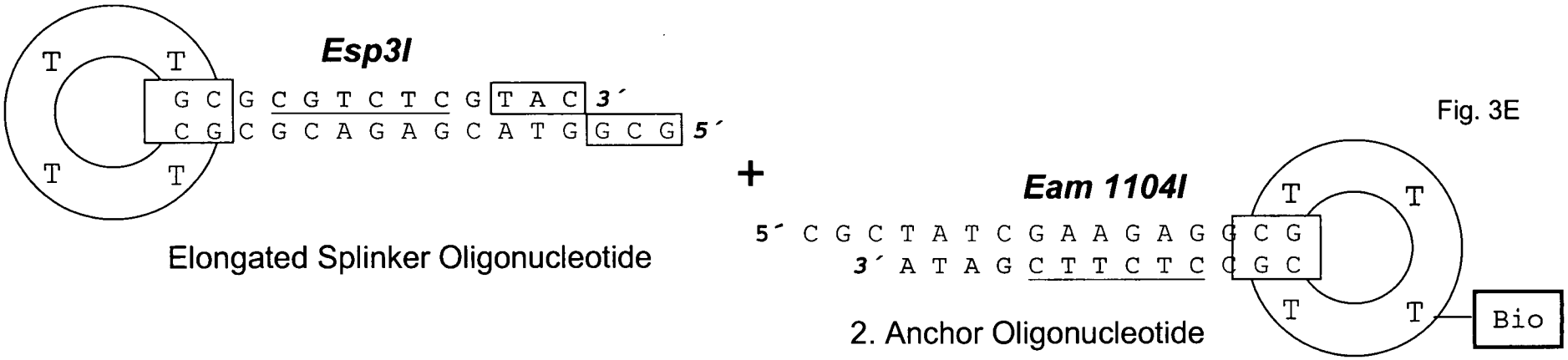


Fig. 4 3nt Overhang elongation (RLPS Variant 1)

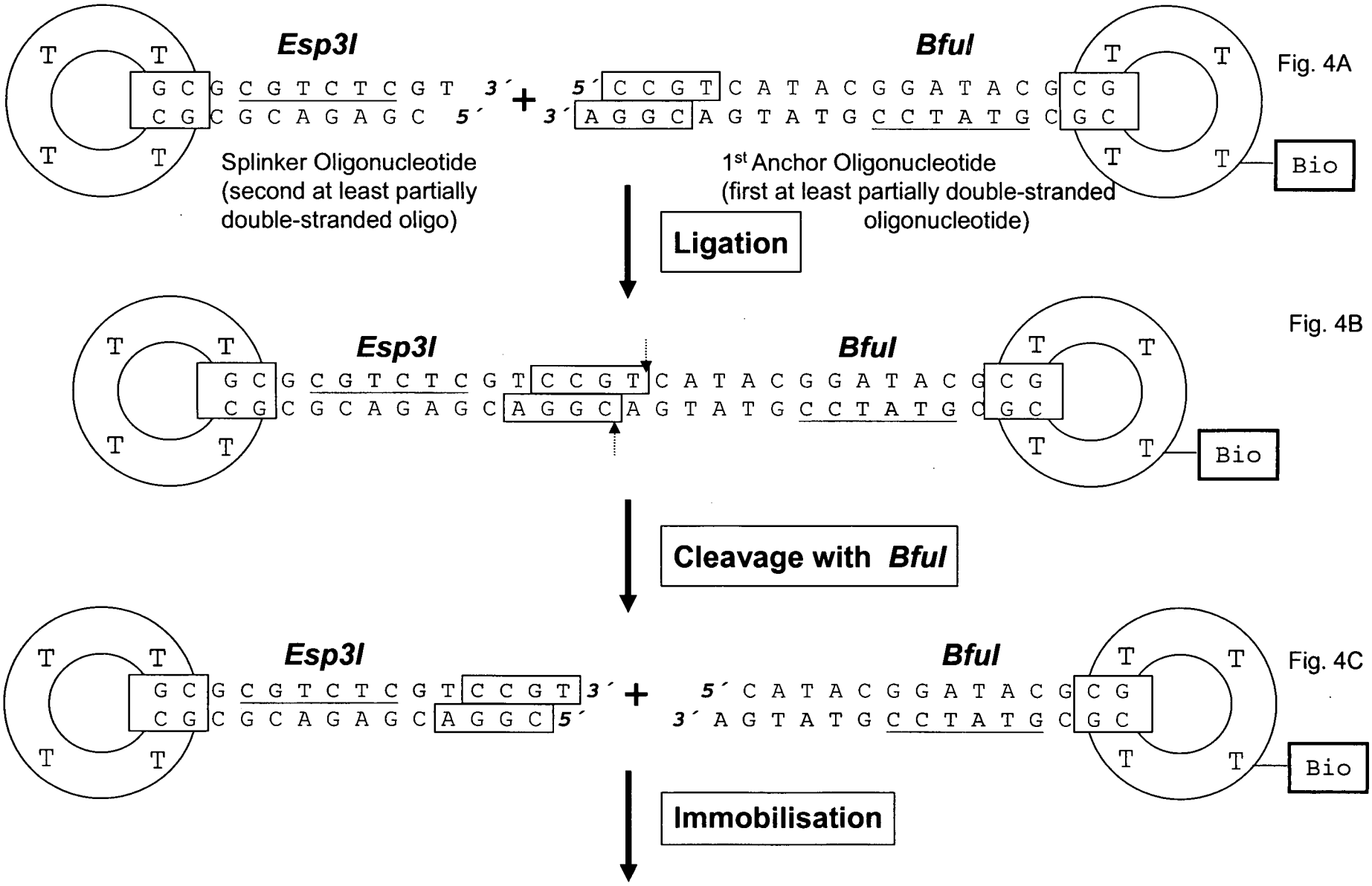


Fig. 4A

Fig. 4B

Fig. 4C

Fig. 4D

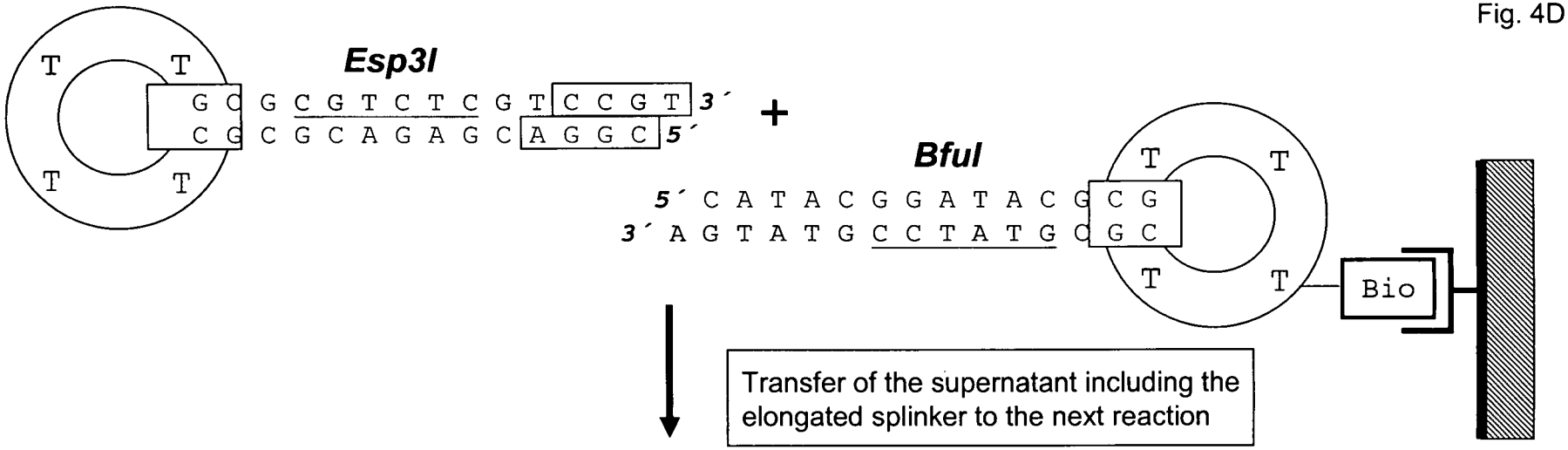


Fig. 4E

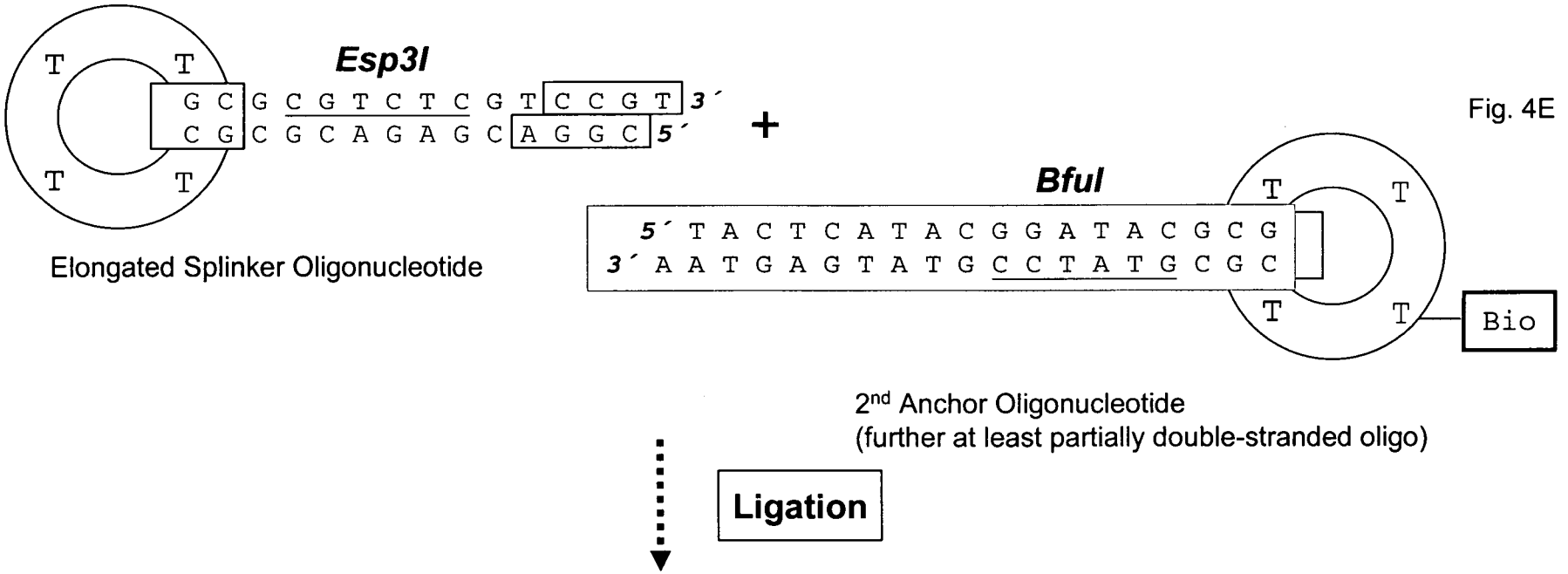


Fig. 5 Addition of transition anchor (both RSPS and RLPS) and first transposition (3 nt overhang)

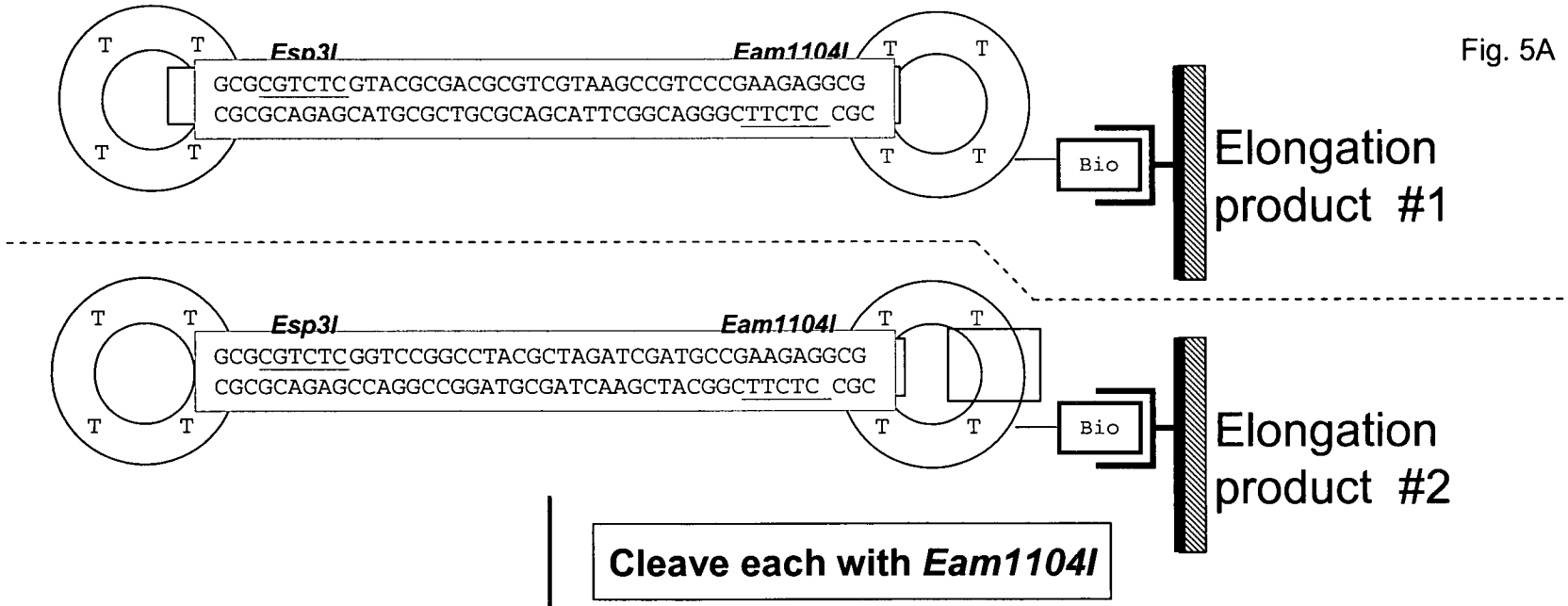
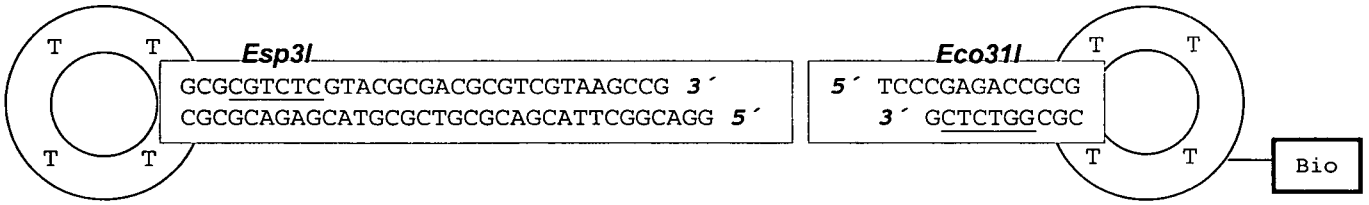


Fig. 5A

Fig. 5B

Cut elongation product #1 with 3 nucleotide overhang at 5' end

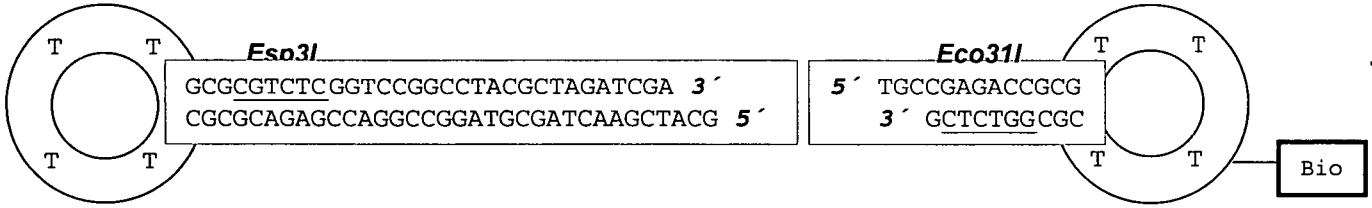
Cut elongation product #2 with 3 nucleotide overhang at 5' end



Transition #1

Fig. 5C

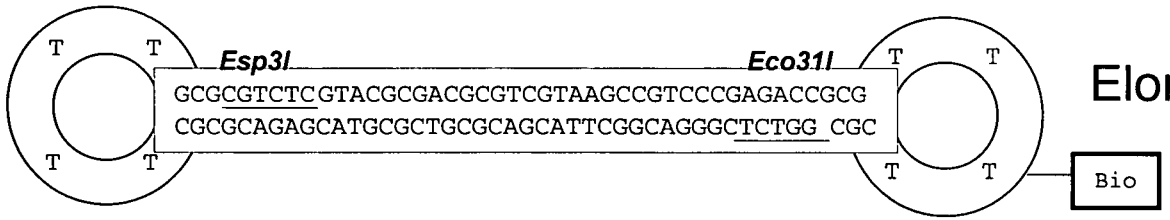
Transition anchor #1



Transition #2

Transition anchor #2

Ligation



Elongation block #1

Fig. 5D

Elongation block #2

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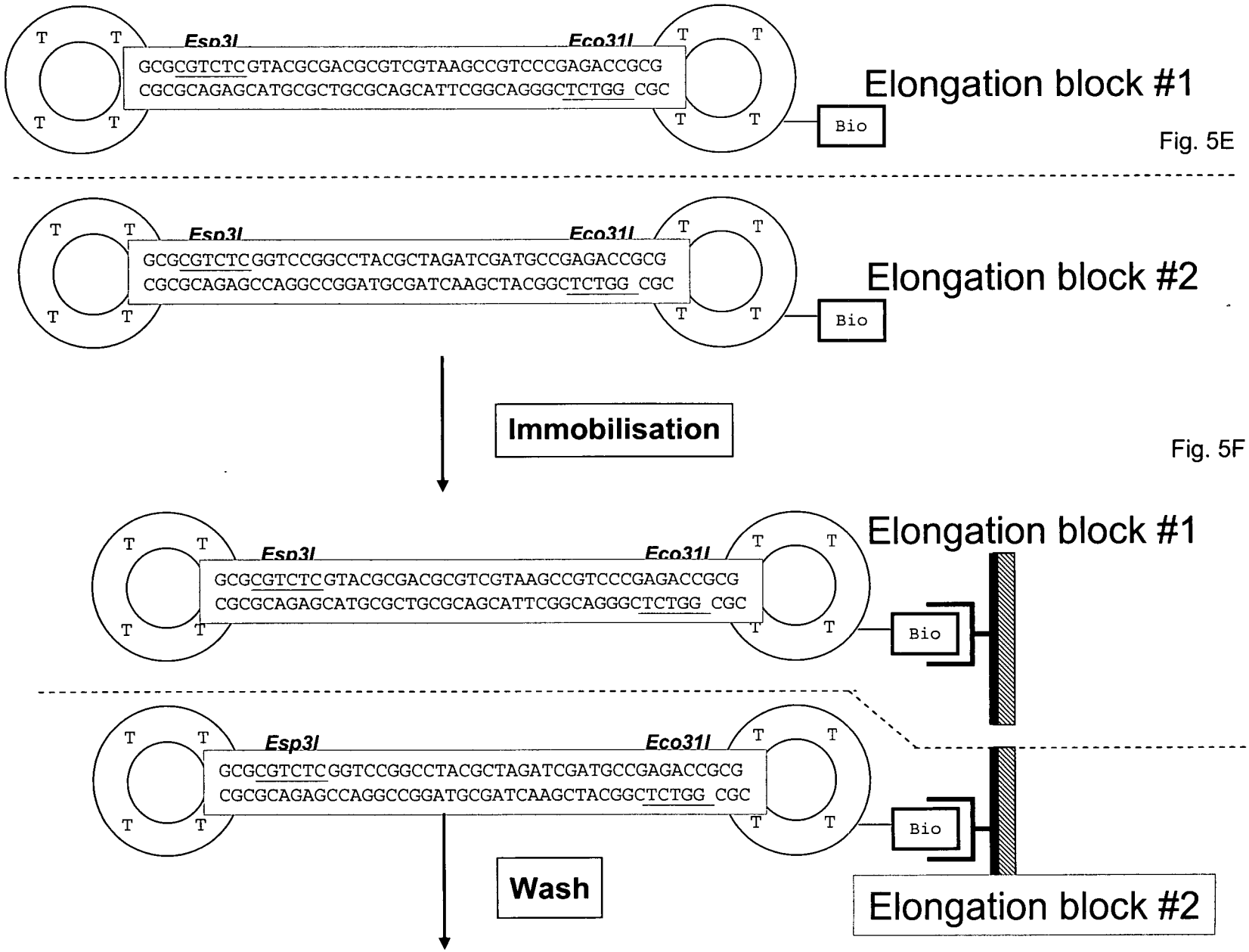
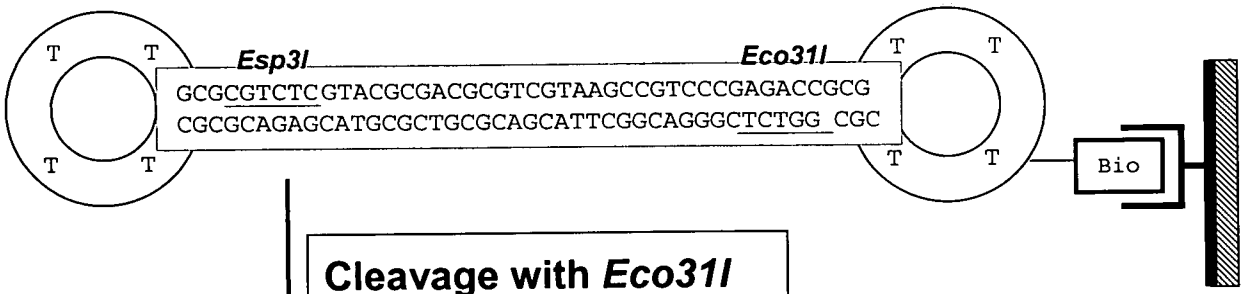


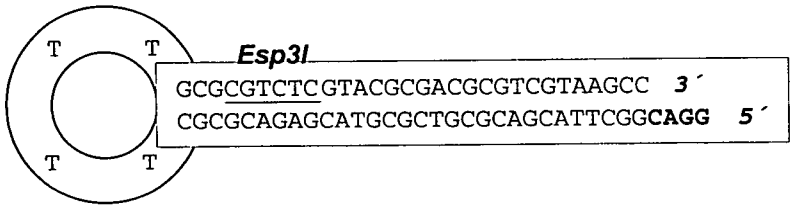
Fig. 5E

Fig. 5F



Elongation block #1

Fig. 5G



Eco31I cut Elongation block

Elongation block #2

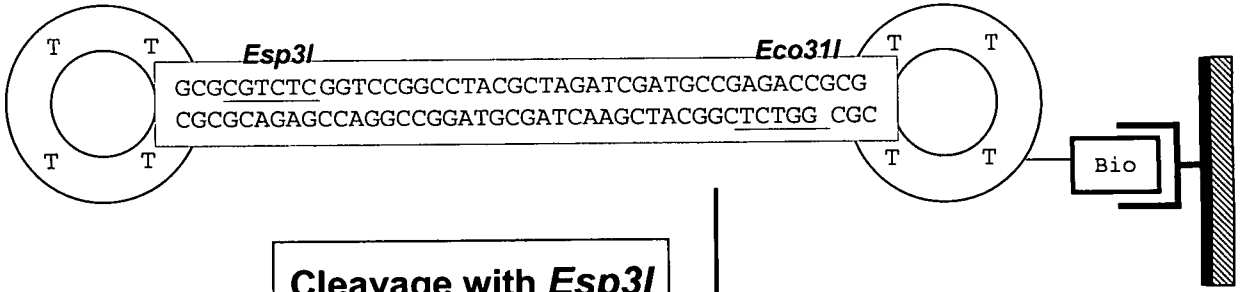
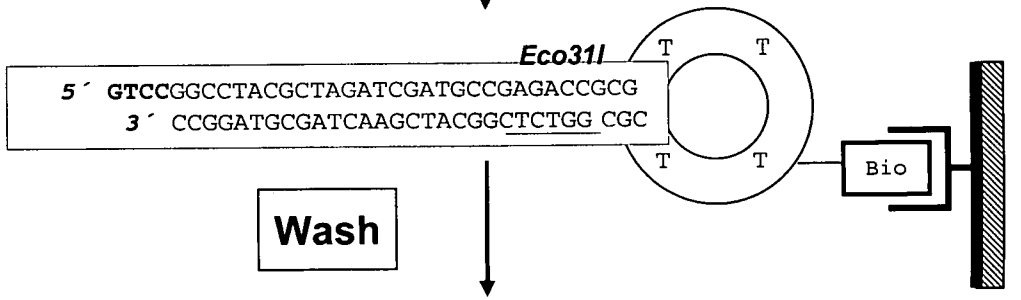


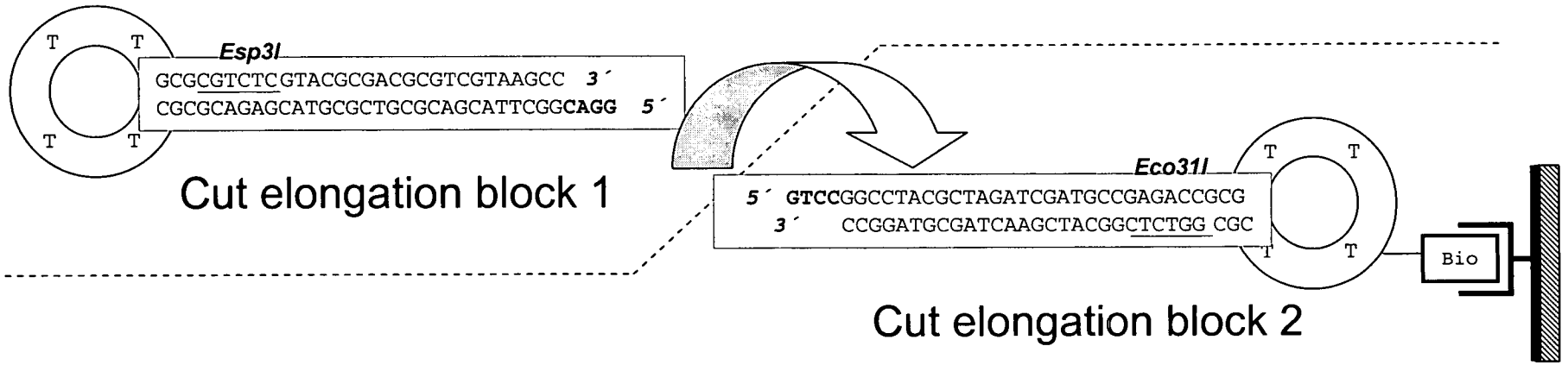
Fig. 5H

Elongated transition anchor



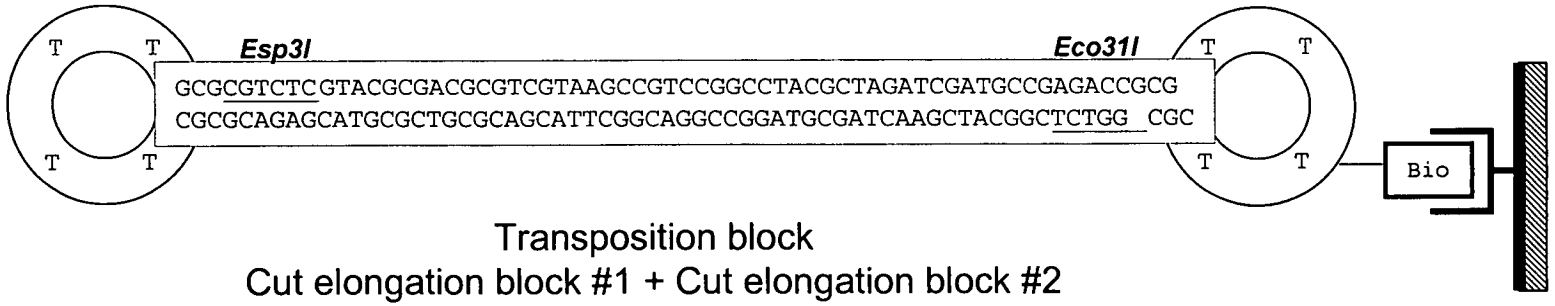
Transfer supernatant with cut elongation block from elongation #1 to an elongated transition anchor

Fig. 5I



Ligation

Fig. 5J



Transposition block
Cut elongation block #1 + Cut elongation block #2

Fig. 6 Addition of transition anchor (both RSPS and RLPS) and first transposition (1 nt overhang)

Fig. 6A

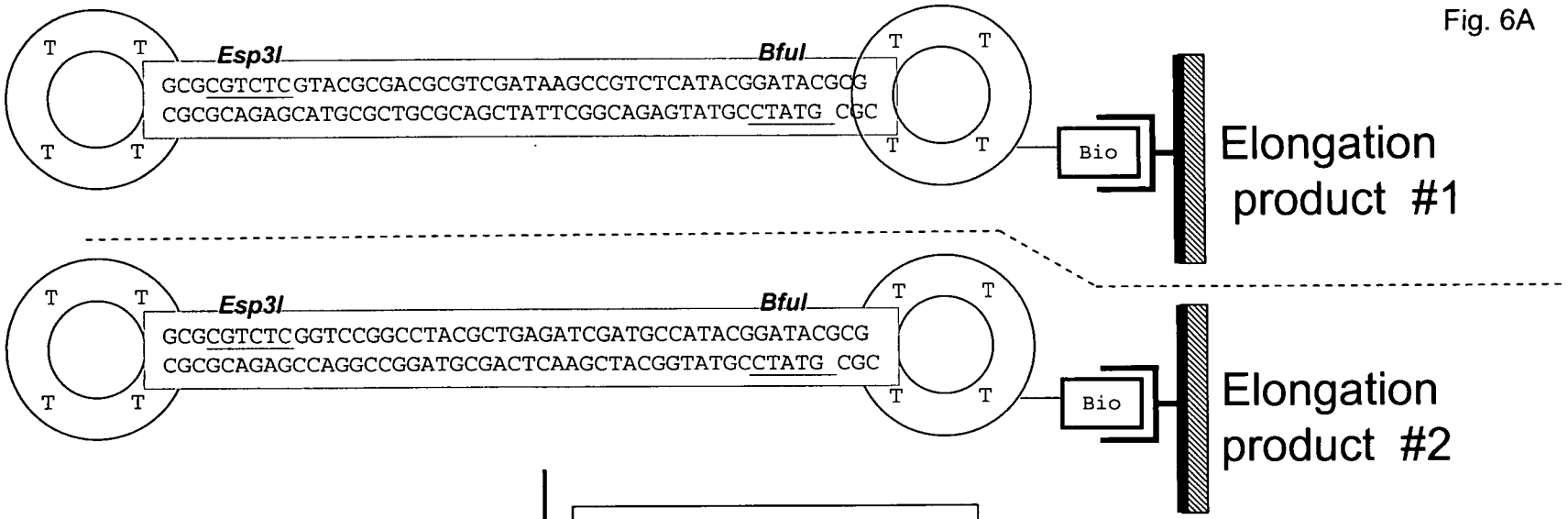
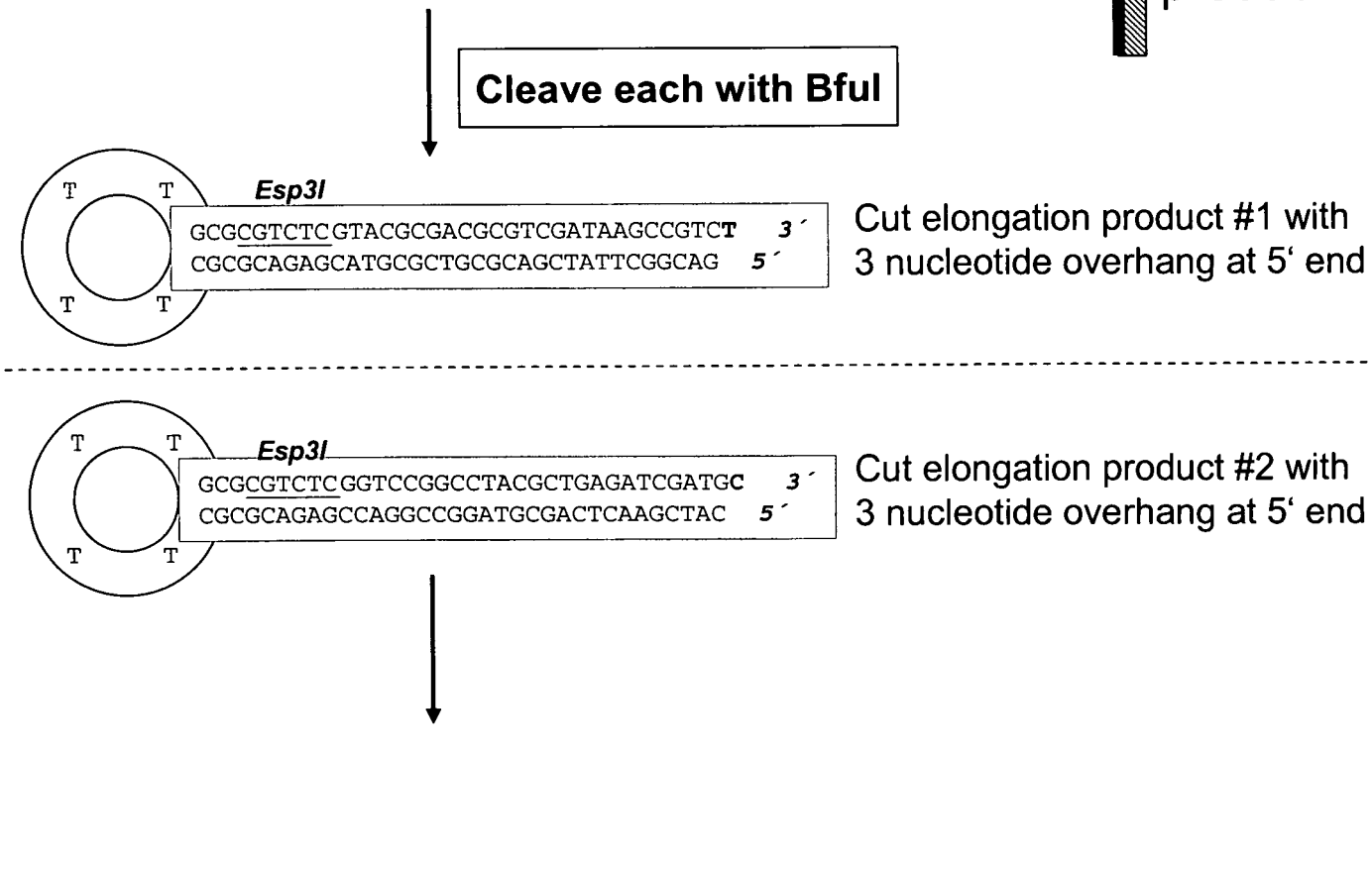
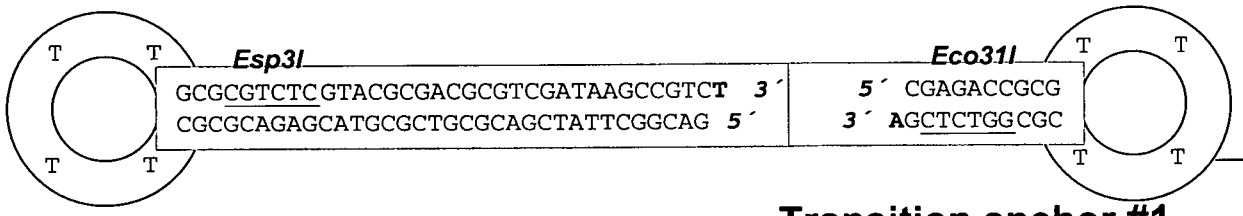


Fig. 6B

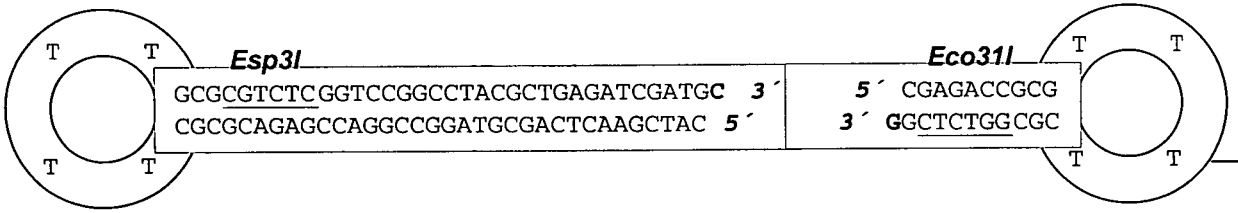




Transition #1

Transition anchor #1

Fig. 6C

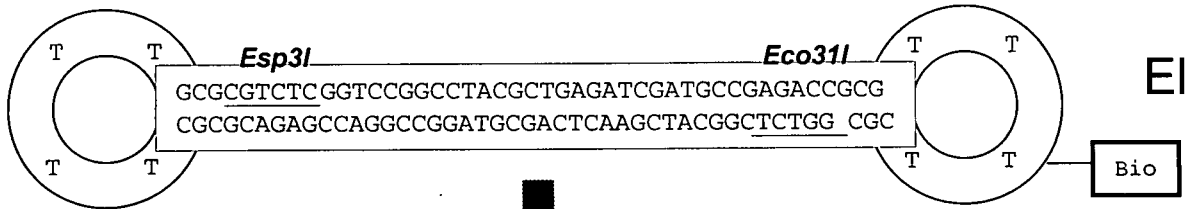


Transition #2

Transition anchor #2

Fig. 6D

Elongation block #1



Elongation block #2

Further steps as in Fig. 5E to 5J

Fig. 7 Semi-Inverted Transposition (SIT).

Fig. 7A

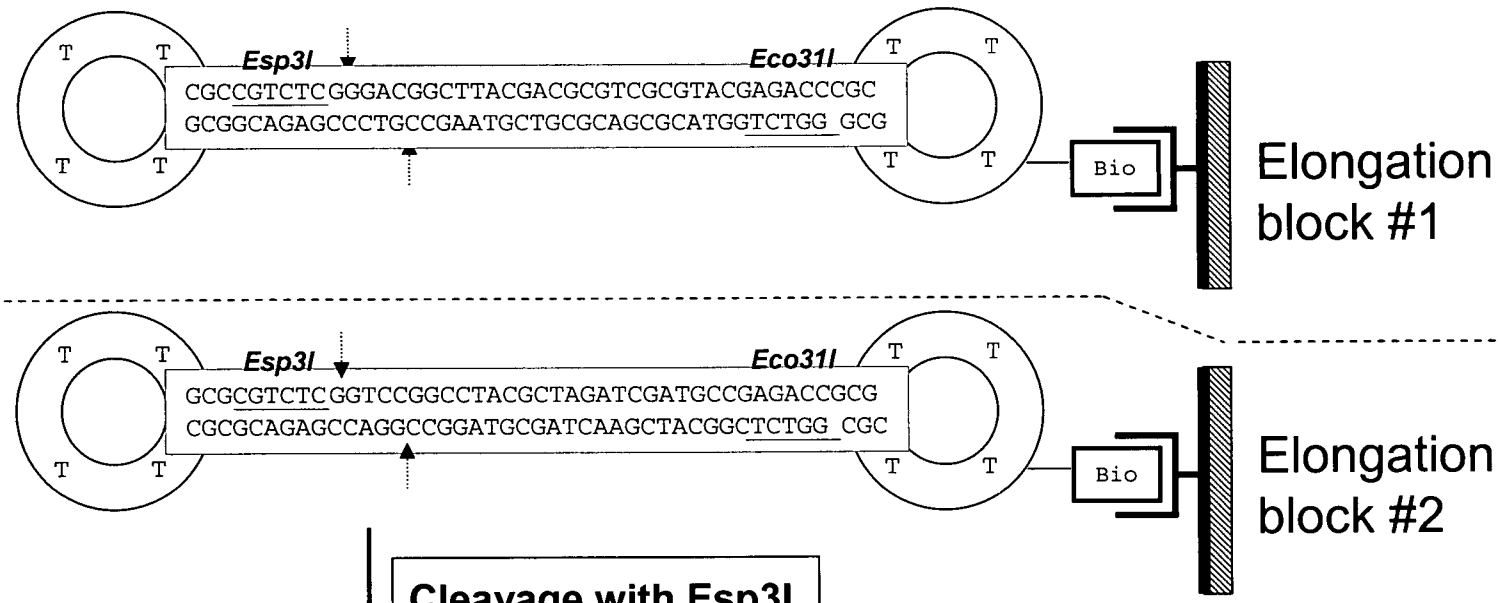
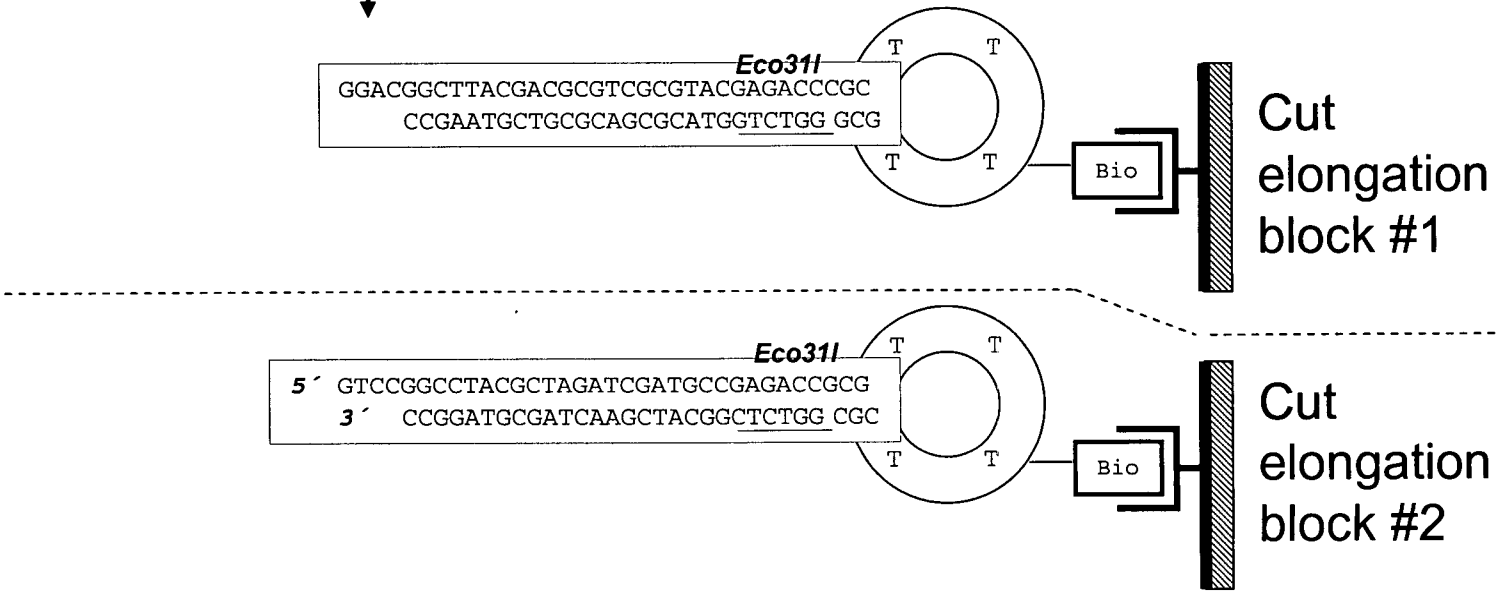
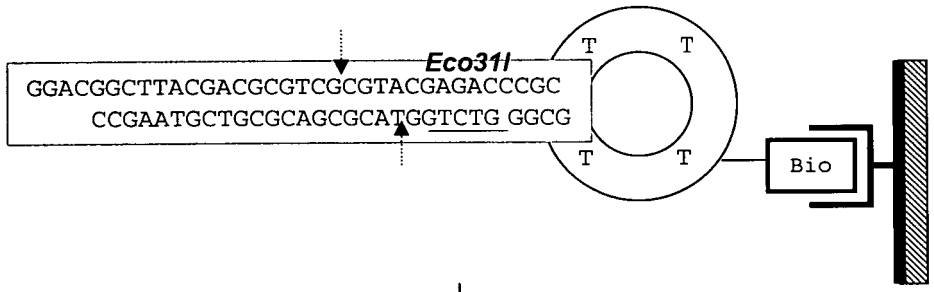


Fig. 7B





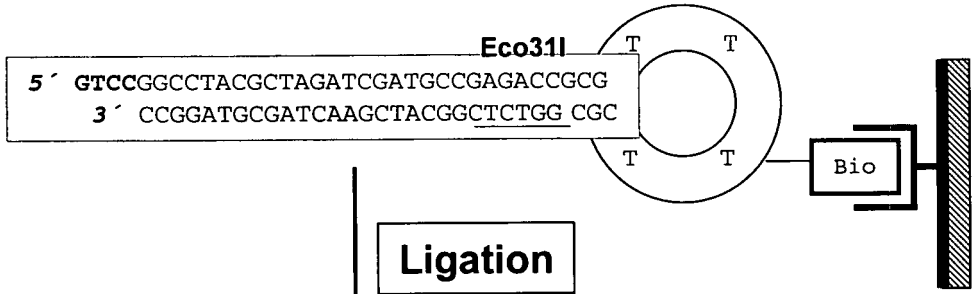
Cut elongation block #1

cleavage of elongation block #1 with *Eco31I*

Fig. 7C

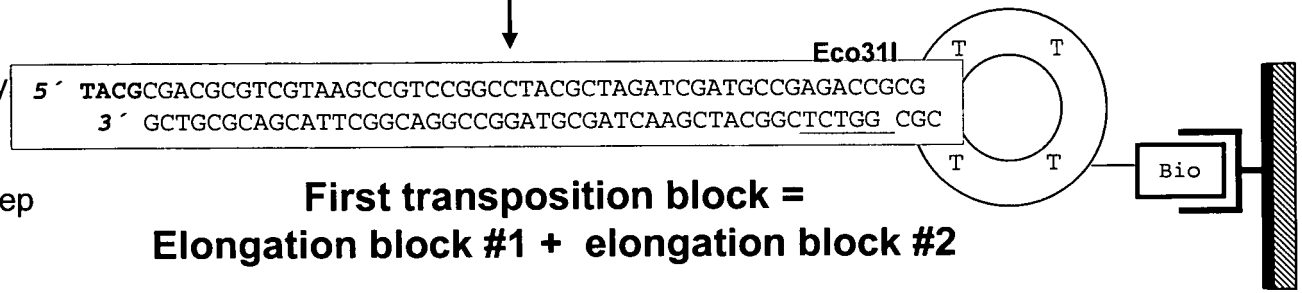
5´ **GGAC**GGCTTACGACGCGTCG 3´
3´ CCGAATGCTGCGCAGCGCAT 5´ linear double cut elongation block

Transfer supernatant with the linear double cut elongation block #1 to elongation block #2



Cut elongation block #2

Ligation



Complementary overhang for subsequent transposition step

First transposition block = Elongation block #1 + elongation block #2

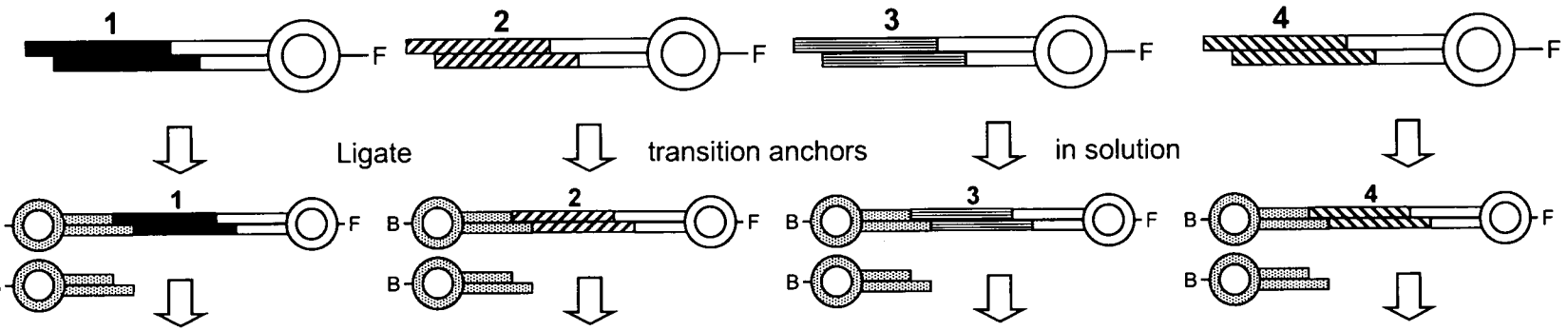
Fig. 7D

Double selection or pingpong procedure

Fig. 8A

Standard elongation reactions with FITC-labelled splinkers and Biotin-labelled anchors

Purified elongation products before addition of transition anchor (last further at least partially double-stranded oligonucleotide)



Immobilise on α-FITC, wash | Immobilise on SA, wash | Immobilise on α -FITC, wash | Immobilise on SA, wash

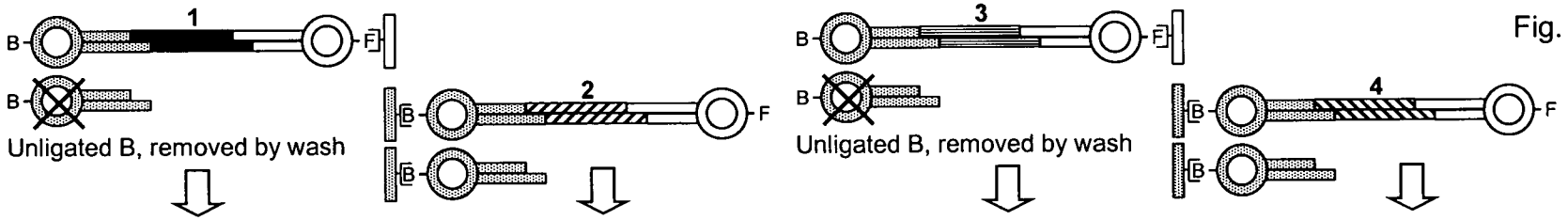


Fig. 8B

Cleave with Esp3I, Transfer supernatant | Cleave with Eco31I, Transfer supernatant | Cleave with Esp3I, Transfer supernatant | Cleave with Eco31I, Transfer supernatant

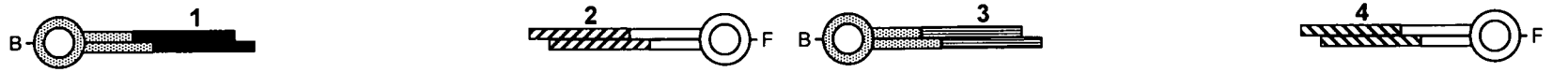
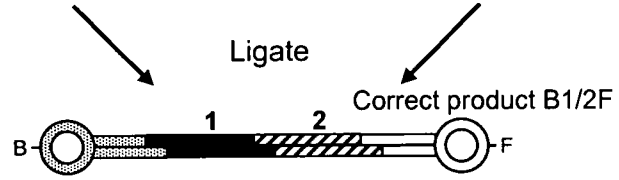


Fig. 8C



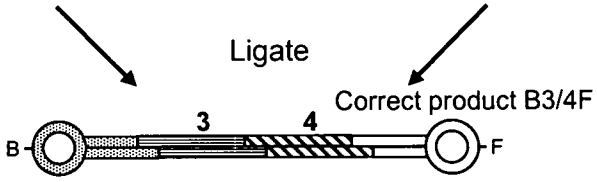
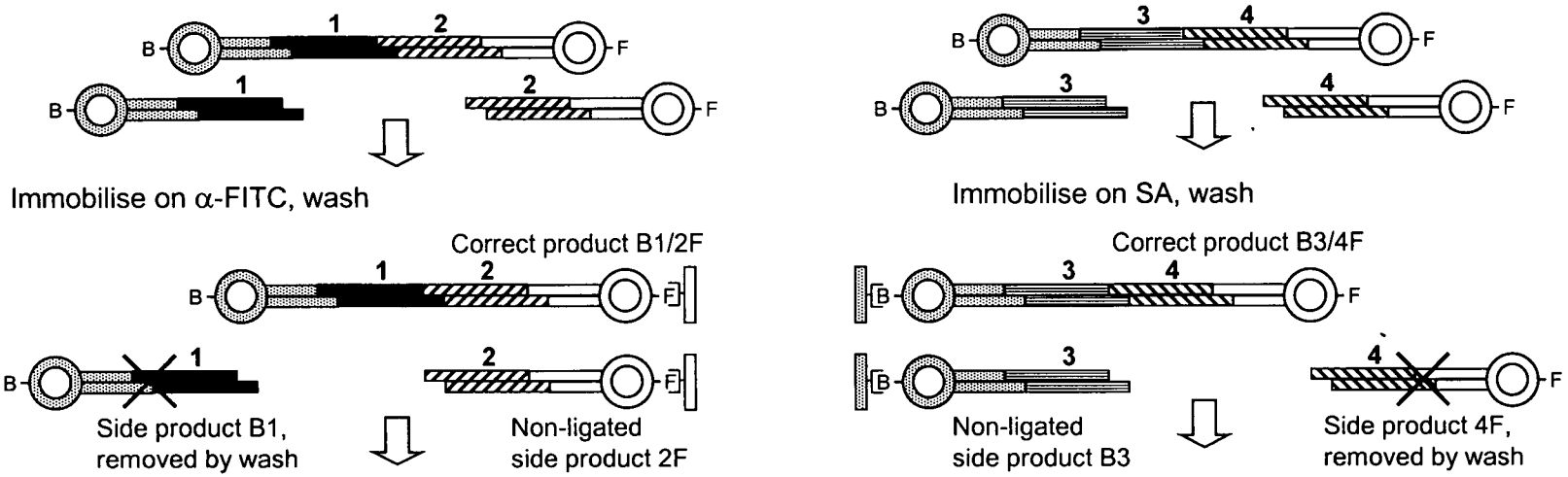


Fig. 8D

Double selection or pingpong procedure

Fig. 8E



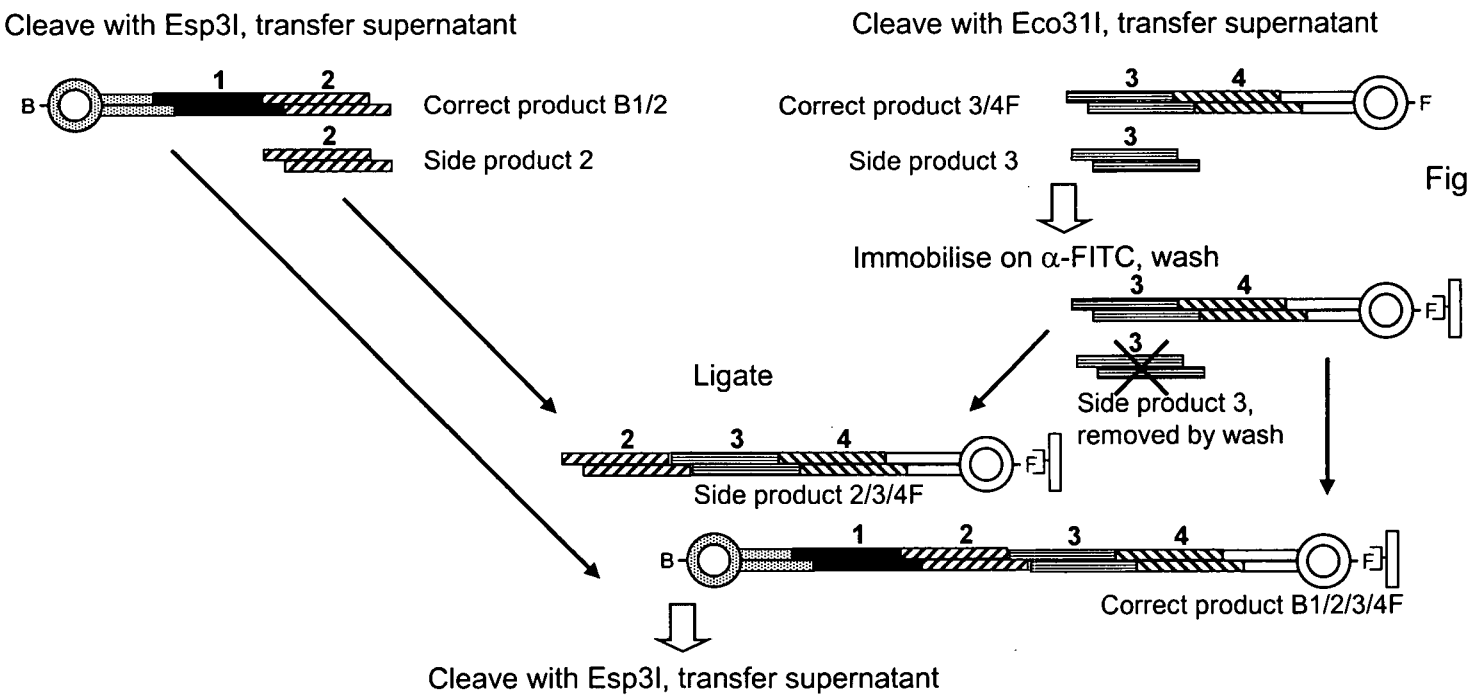
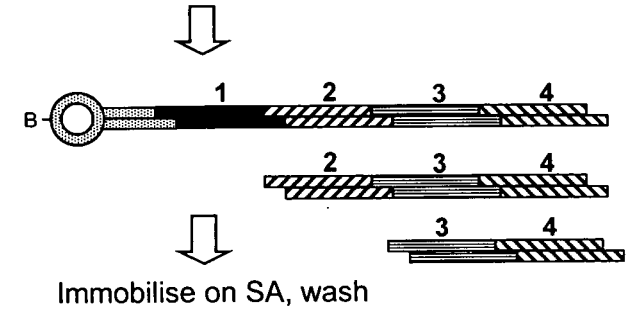


Fig. 8F

Double selection or pingpong procedure

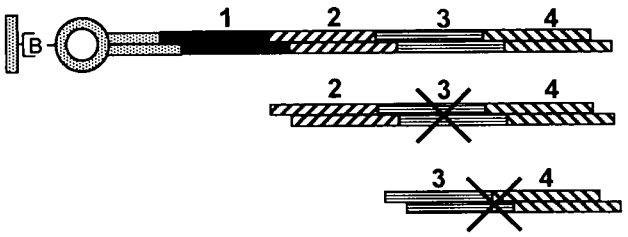
Fig. 8G



Correct product B1/2/3/4

Side product 2/3/4 arising from ligation with 2

Side product 3/4 arising from non-ligated 3/4F

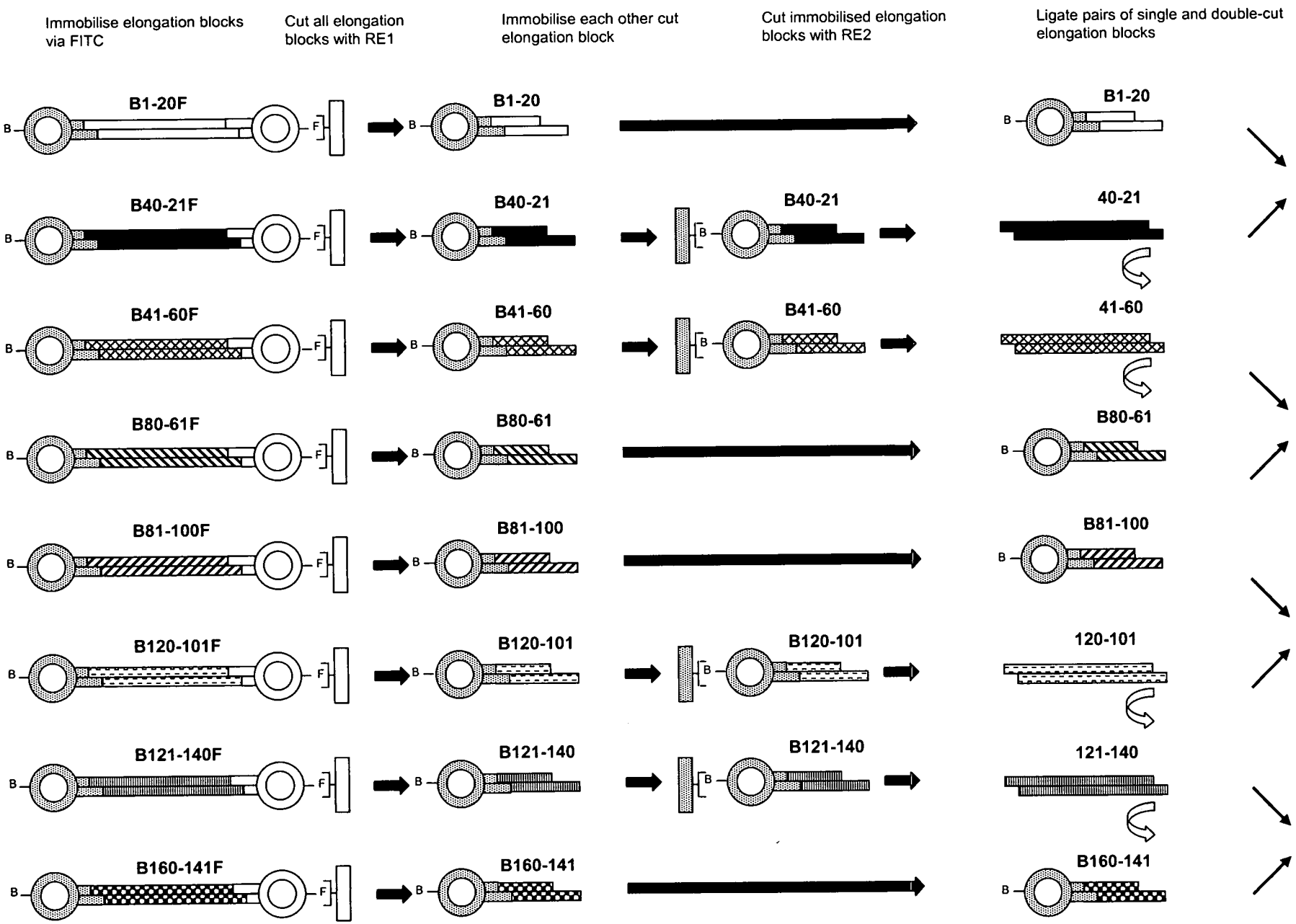


Side product 2/3/4 removed by wash

Side product 3/4 removed by wash

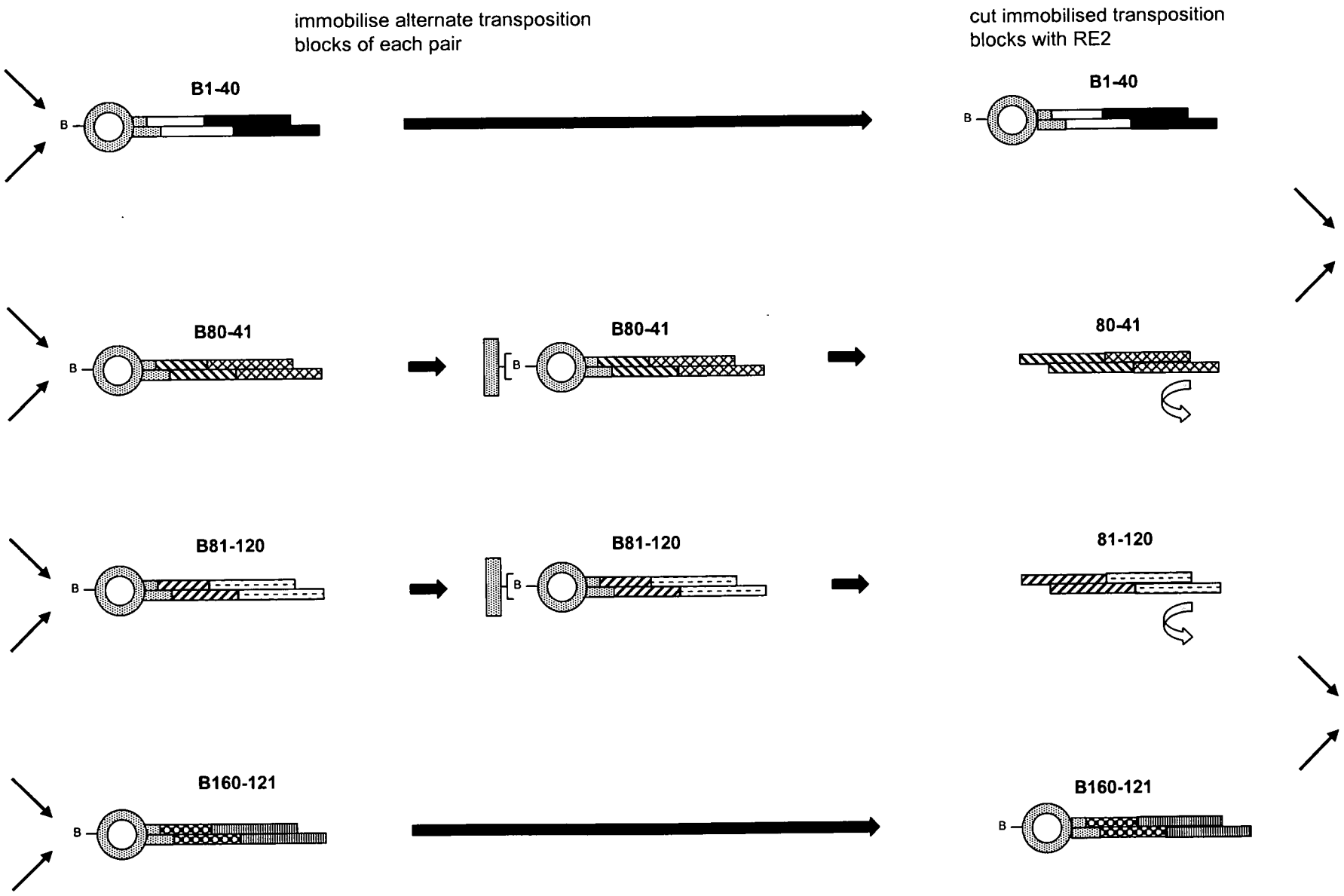
Semi-inverted transposition (SIT) with prior double selection

Fig. 9A



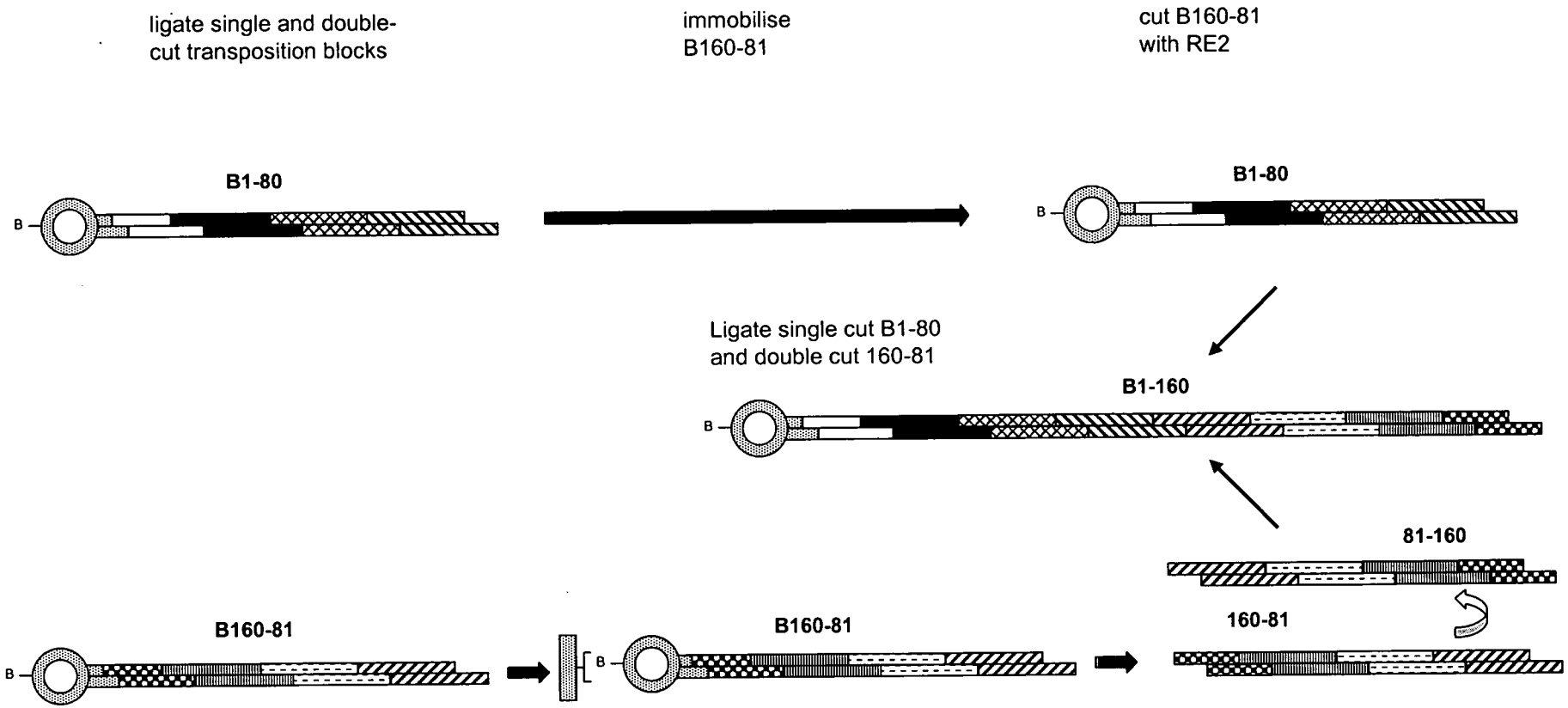
Semi-inverted transposition (SIT) with prior double selection

Fig. 9B



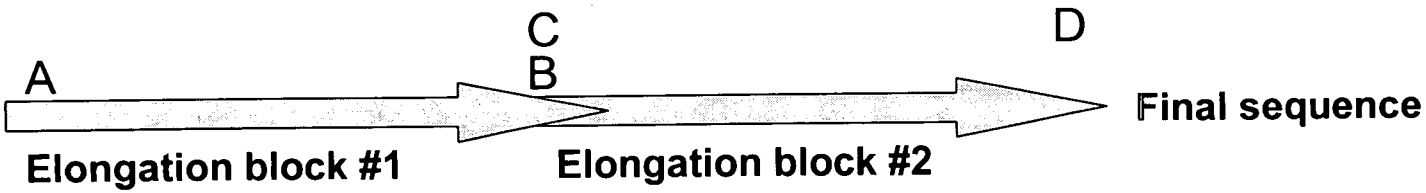
Semi-inverted transposition (SIT) with prior double selection

Fig. 9C

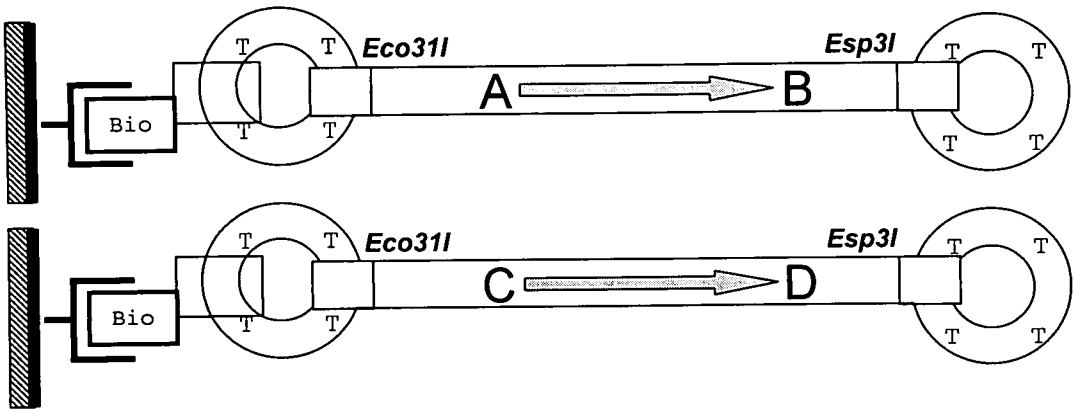


Design of elongation blocks for standard and semi-inverted transpositions

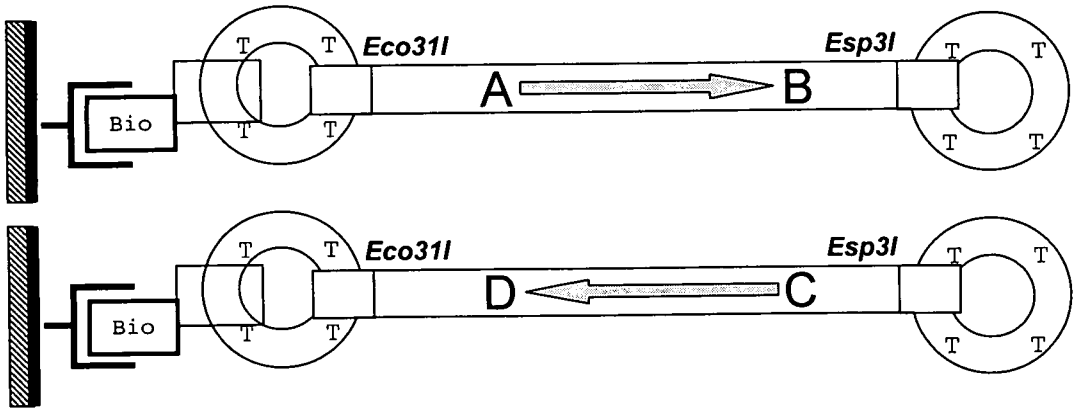
Fig. 10



Design of the Elongation blocks for Standard Transposition



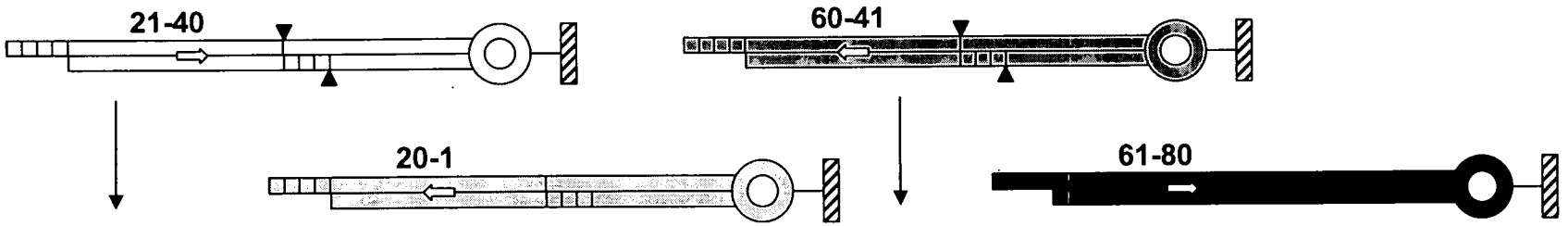
Design of the Elongation blocks for SIT



Semi-inverted transposition (SIT) with 3nt/4nt ligation

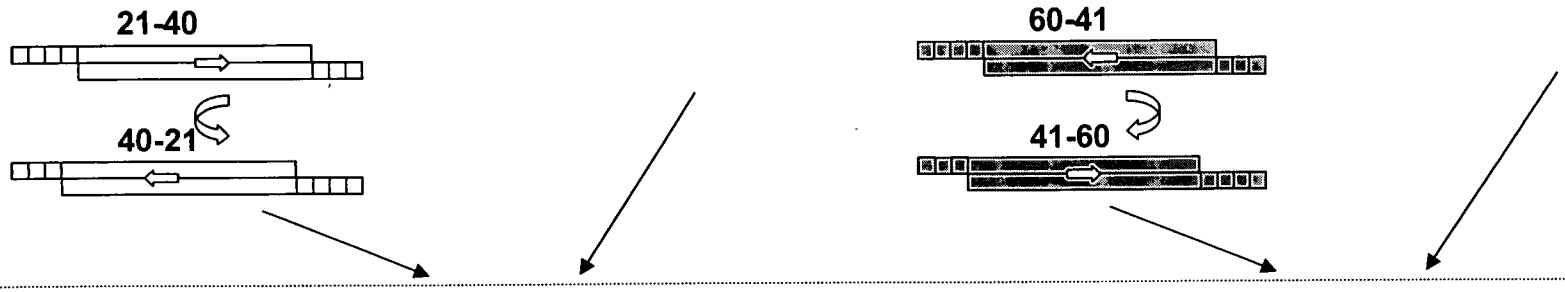
Fig. 11A

1. cleave all immobilised elongation blocks with RE specific for second at least partially double-stranded oligonucleotide



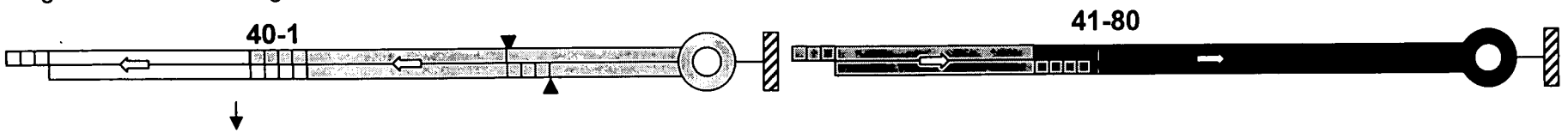
2. cleave every other cut immobilised elongation block with RE specific for further at least partially double-stranded oligonucleotide

Fig. 11B



3. ligate double cut elongation blocks in inverse orientation with the respective matching single cut immobilised elongation blocks

Fig. 11C



4. cleave every other immobilised transposition block with the same RE as before, ligate double cut transposition blocks in reverse orientation with their respective matching single cut immobilised transposition blocks

Fig. 11D

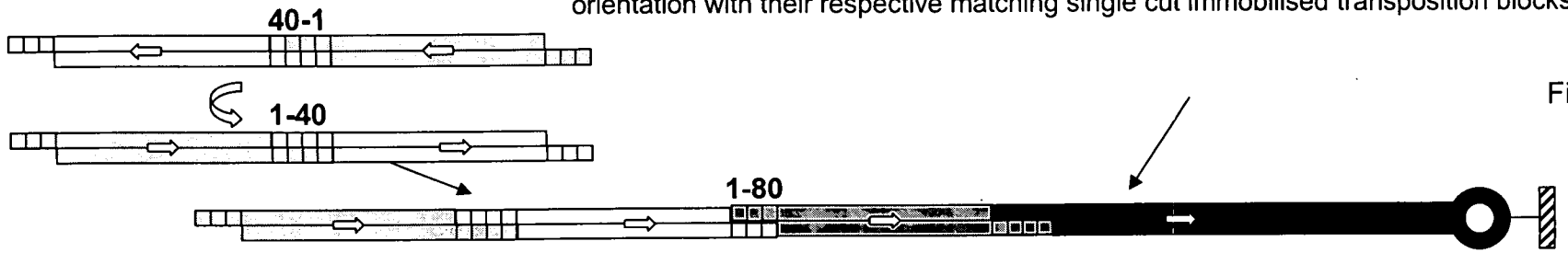


Fig. 12

SPS (solid phase synthesis)

RLPS (reverse liquid phase synthesis)